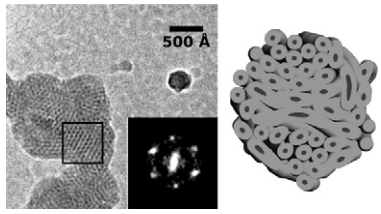
exhibit a local hexagonal packing and evolve from disordered agregates to spooled then entangled straight bundles as the rigidity of lipid-coated polyelectrolytes increases. These assemblies may constitute a generic route for interfacing polyelectrolytes to living cells like in gene delivery.



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Structure, Assembly and Mechanical Properties of DNA Nanoparticles Condensed with Peim

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DNA is an anionic polyelectrolyte, which occupies a large volume in salt free solution due to the coulomb repulsion between the charged groups. In the presence of polymer cations, DNA condenses into nanoparticles. DNA nanoparticles have generated a lot interest as a preferred vehicle for delivering therapeutic DNA in gene therapy. The efficiency of gene delivery is determined by stability and compactness of the particles. However, not much is known about the organization of DNA within the nanoparticles. Large polymer cations condense DNA rapidly, with no distinct intermediate stages that could give insight into the arrangement of DNA. In our work, we modulate the DNA length to slow down nanoparticle formation; and, by imaging with Atomic Force Microscopy, reconstruct stages in the particle assembly. The polymer cation used was polyethyleneimine modified with sugar residues (PEIm). The cation:base pair ratio was ~60. The DNA is found to be arranged within the nanoparticle as an inter-weaving network of long fiber condensates. The fiber condensates form from DNA folding along its length, and appear to be the unit of DNA organization within the particle. The fiber-condensate network is highly deformable, having as much as 95% water content. Nano-indentation experiments suggest that the nanoparticles have a hollow sphere architecture.

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Extracellular Matrix Proteins are Necessary for Mouse Embryonic Stem Cell Differentiation and May Guide Stem Cell Fate

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While in vitro differentiation protocols often rely exclusively on soluble growth factors to direct mouse embryonic stem cell (mESC) fate, the ESC niche contains both soluble factors and fibrillar extracellular matrix (ECM) proteins, including fibronectin (FN). Moreover, some of the soluble factors used to guide differentiation (e.g. Activin A) are known to increase the expression of ECM proteins, though the functional importance of this change is not well understood. We examined whether ECM proteins were necessary for promoting and directing mESC differentiation. mESCs, grown as embryoid bodies under differentiating conditions in the absence of FN, maintained expression of the pluripotency marker, Nanog. The embryoid bodies also showed a spatiotemporal correlation between expression of FN and GATA4, a marker for differentiation. When differentiated on a gelatin substrate, mESCs create a fibrillar ECM containing fibronectin and laminin components, while the presence of leukemia inhibitory factor (LIF), a maintainer of mESC pluripotency, inhibits the production of this fibrillar matrix. Ongoing work is investigating how the composition of this cell-derived ECM changes when soluble growth factors are used to guide ESC fate and whether these changes are necessary to efficiently direct differentiation. Together these data imply that FN is necessary for mESC differentiation and that the extracellular matrix may be an important director of stem cell fate.

3645-Pos Board B506

Investigating the Effects of Dynamic External Stimuli on Single Cell Fitness and Gene Expression in Escherichia Coli

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In this work, we study the bacterial cellular response to time-dependent external stimuli in single living cells. We developed a microfluidic platform for single cell analysis that allows for dynamic control of well-defined environmental growth conditions and culture media. Using this platform, we studied the effect of small molecule inducers on gene expression in the *lac* operon using fluorescent reporter proteins and cell growth rates as a proxy of cellular fitness. We applied temporally varying inducer concentrations by translating single cells between two adjacent fluid streams containing either growth medium or growth medium and inducer. We observed that single cell gene expression depends on growth rate and frequency of exposure to inducer concentrations. Single cell induction experiments are compared to control experiments with and without continuous fluid flow in microfluidic channels. For these experiments, single cell analysis is facilitated by a microfluidic-based hydrodynamic trap recently developed in our lab. The hydrodynamic trap enables confinement and manipulation of single cells in free solution using the sole action of fluid flow. Automated feedback control is integrated into the device using an ''on-chip'' valve, which allows for precise confinement of cells in free solution. Using this device, cells are confined at a fluid stagnation point generated in a cross-slot microfluidic geometry, thereby enabling non-perturbative trapping of cells for long time scales. Using optical microscopy, we observe single *Escherichia coli* cells growing and dividing both at room temperature and at 37^0C, and cell division rates in the microfluidic trap compare favorably to the growth rates of *E. coli* measured in bulk studies. We anticipate that the microfluidic-based trap is an ideal platform to study cellular regulation and gene network dynamics of single living cells in free solution.

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Infrared Light Excites Cells via Transient Changes in Membrane Electrical Capacitance

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Control of excitable cells using optical technologies such as optogenetics has enabled important advances in neuroscience and the development of clinical applications. Most existing methods of optical control require the use of genetic or chemical sensitizers that enable light to alter the ionic conductance of cell membranes. By contrast, infrared (IR) light of wavelengths $> 1.5\mu m$ has been shown in vivo to excite neural and muscle tissue without any pretreatment. Unfortunately, the mechanism of IR stimulation is unknown. Here, we describe how IR light excites cells by transiently altering their membrane electrical capacitance. Our data from voltage clamped Xenopus laevis oocytes, mammalian cells and artificial lipid bilayers shows that IR energy absorbed by water produces a rapid local increase in temperature at the cell membrane, transiently increasing its electrical capacitance, and generating depolarizing currents. Correspondingly, under current clamp conditions, IR pulses produce rapid changes in membrane potential. This unexpected mechanism is fully reversible and requires only the most basic properties of cell membranes. Changes in capacitance were verified by direct measurement in mammalian cells and artificial bilayers, and are consistent with a classical theoretical description of cell membranes as coupled double-layer capacitors. In shedding light on the mechanism of IR stimulation, our findings point to this technology's unique generality as a means to control excitable cells, and raise questions about other thermal phenomena that may meaningfully affect membrane electrostatics. Supported by the NIH: GM030376 and DC011481-01A1.

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Mechanical Derivation of Functional Myotubes from Adipose-Derived Stem Cells

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In recent years, ECM stiffness and resulting cell contractility have been identified as potent stem cell differentiation regulators. Successful stem cell-based therapies will require acclimating cells to the abnormally stiff ECM of muscular dystrophy while inducing and/or maintaining myogenesis, fusion, and dystrophin delivery. Here we directly compare ASC to BMSC stiffness responsiveness and show myotube formation derived from ASCs on matrices that mimic skeletal muscle. ASCs are shown here to not just simply reflect the qualitative stiffness sensitivity of bone-marrow-derived stem cells (BMSCs) but to exceed BMSC myogenic capacity (40-fold higher myogenic marker expression on myogenic stiffness), expressing the appropriate temporal sequence of muscle transcriptional regulators on muscle-mimicking extracellular matrix in a focal adhesion- and contractility-dependent manner. 2% of ASCs formed multi-nucleated myotubes with a continuous cytoskeleton (10-fold higher than chemical induction) that was not due to misdirected cell division; microtubule depolymerization severed myotubes, but after washout, ASCs refused at a rate similar to pretreated values. BMSCs never underwent stiffness-mediated fusion. ASC-derived myotubes, when replated onto non-permissive stiff matrix, maintain their fused state. Fusion frequency was increased by a contractile agonist, lysophosphatidic acid and decreased by a myosin inhibitor, blebbistatin. ASCs generated higher tangential force than BMSCs and